PRODUCTION OF COAGULASE BY STAPHYLOCOCCI

BY

CLARENCE VERNON HUBBARD

A. B., University of Iowa, 1936

M. S., University of Illinois, 1945

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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PRODUCTION OF COAGULASE BY STAPHYLOCOCCI

The ability of staphylococci to coagulate blood plasma has been recognized and studied at intervals since 1903. Loeb (1903) reported that certain cultures of *Staphylococcus aureus* had the ability to coagulate goose plasma. Much (1909) described the phenomenon in detail and was the first to suggest that there was a relationship between production of coagulase and the pathogenicity of the strain in question. Daranyi (1926) and Gross (1928, 1931) reported that the production of coagulase is a good indication of pathogenicity. Since then this has been confirmed by many workers, and at present the coagulase test has become a routine procedure in many clinical laboratories and is used as a means of identifying pathogenic staphylococci.

A number of media were prepared and sterilized in flasks. Each flask was inoculated with one ml. of a 24 hour broth culture of *Staphylococcus aureus* strain #9. They were incubated at 37°C. and at intervals coagulase tests were run to determine coagulation time. The results obtained showed that Nutrient broth is not a good medium for coagulase production. Media containing dextrose produce very small amounts of coagulase. Probably because the pH of the culture gets as low as 4.4. Brain heart infusion broth, Heart infusion broth, and Tryptose phosphate broth, give good production of coagulase. Broth containing 20% plasma produced more coagulase than any of the other media. More active cultures were obtained in test tubes than when the cultures were grown in flasks. Thirty percent carbon dioxide slightly decreased the amount of coagulase produced.

There is a quantitative relationship between the amount of coagulase present and the time required for plasma to clot. The coagulation time is also affected by the age of the plasma used. Oxalated rabbit plasma may be stored in the refrigerator for as long as seven weeks and still give

good results. However the time required for coagulation gets longer as the plasma increases in age. Dried Bacto-Coagulase-Plasma is as good as rabbit plasma except that slightly longer times are required for coagulation.

The coagulase active substance was concentrated by precipitation with 50% ammonium sulphate, eight volumes of ethyl alcohol, and by adjusting the pH to a value of 3.5. By precipitation with 50% ammonium sulphate and redissolving the precipitate formed in 1/10 the volume of distilled water and then adsorbing with Norite an active concentrate was prepared which reduced the original coagulation time of 90 minutes to 5 minutes. Adsorption with Norite removed all colored material as well as the fibrinolytic material present in the filtrate from this particular culture. The unadsorbed concentrated filtrate clotted plasma in 5 minutes, but the fibrinolytic substance dissolved the clot in less than 24 hours. The adsorbed concentrated filtrate failed to dissolve the clot in more than a week.

An active precipitate formed by alcohol precipitation was dried in a vacuum and stored at room temperature. It was still active and able to coagulate plasma when diluted to the original volume after a period of six months.

Brain heart infusion broth cultures of *Staphylococcus aureus* strain #4 were grown for 72 hours and filtered through Mandler, Seitz, Berkefeld, and Pyrex sintered glass UF filters. The filtrates were tested for coagulase activity and all but the filtrates from the Seitz filter were found to be coagulase positive. Brain heart infusion broth cultures were grown from 34 strains of coagulase positive Staphylococci. These were filtered through Pyrex sintered glass filters and the filtrates tested for coagulase activity. Four of the 34 cultures failed to give a coagulase positive filtrate.

Coagulase positive staphylococci were grown in mixed culture with 59 stock cultures of other bacteria available. Coagulase tests were run on the mixed cultures. When the

staphylococcus was a weakly coagulase positive culture, about one third of the stock cultures tested inhibited the production of coagulase. When the stahpylococcus was a strongly coagulase positive culture, about one twelfth of the stock cultures inhibited coagulase production. The organisms most often inhibiting coagulase production were gram negative, non-spore forming rods.

Besides staphylococci the only other organisms encountered that was able to clot plasma was a culture of Serratia marcescens, and it required somewhere between 24 and 48 hours to form a clot in plasma.

VITA

The author was born at Seneca, Wisconsin, on March 13, 1909. He attended the elementary and secondary schools in Carroll, Iowa, and graduated from Carroll High School in 1928. The Bachelor of Arts degree was received from the University of Iowa in June, 1936. The Master of Science degree in Bacteriology was conferred by the University of Illinois in June, 1945. From 1937 to 1944 he was employed as bacteriologist by Morrison Clinic, Carroll, Iowa. From October, 1944, until June, 1947, he was an assistant in the Department of Bacteriology at the University of Illinois.

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